A COMPARISON OF THE EMBRYOGENY OF PICEA AND ABIES

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Picea Smithiana Boiss. The embryogeny of *Picea Smithiana* Boiss. was observed in the spring of 1936 in trees planted on the campus of Stanford University. Though it was impossible to follow the entire embryogeny, the stages of suspensor formation were observed in considerable detail and this account will serve to present more fully than heretofore the differences between the suspensor of a spruce and that of a fir, which will also be described in greater detail.

Picea Smithiana is a species introduced into cultivation more than a century ago from southern Asia. Its native region is given as Afganistan and the Himalayas. Its embryogeny appears to be very similar to that of *Picea Abies* (L.) Karst., (*P. excelsa*), and several American spruces which the writer has examined previously and is probably typical for the genus *Picea.*

The development of the proembryo was not observed. Miyake (7) gave us a description of the proembryo of *Picea excelsa* in all stages. Another record in the form of drawings made nearly a century ago by Schacht (8) describing the late stages in the proembryo of *Picea glauca* (Moench) Voss has been generally overlooked because it was given under the name *Abies alba* Michx., a synonym of *Picea glauca.*

Four tiers of four cells each are found in the proembryo. *Pinus* and nearly all genera of Pinaceae (Abietineae) have this same type of proembryo (2, 4). The uppermost tier of cells is incompletely surrounded by walls, and these four nuclei soon disappear. The uppermost tier of walled cells is the rosette, the name given to this structure by Schacht. Below this is a tier of suspensor cells which elongate in unison, and push the embryonic tier forward into the female gametophyte. The embryonic tier divides very soon after the elongation of the suspensor has begun.

This precocious division of the embryonic cell to form an additional tier is a feature wherein the spruce differs from the fir. In *Abies* the tier of embryonic cells may remain undivided for a considerable period, during which the suspensor becomes very long.

Figure 1 shows the earliest stage of the embryo of *P. Smithiana* obtained from dissections. The division of the embryonic cells into two tiers has already taken place, although the cells of the suspensor tier have only become elongated in the ratio of about 6 : 1. Figure 2 shows a later stage in which the four lowest cells have divided again, so that three cells are now replacing each of

Explanation of the Figures. Plate 15.

Plate 15. Successive Stages in Embryogeny of Picea Smithiana. Figs. 1–6, × 60 (fig. 2 an optical section). s = primary suspensor, e = tier of embryonic cells; e_1, e_2, e_3 = successive tiers of embryonal tubes that constitute secondary additions to the suspensor system.

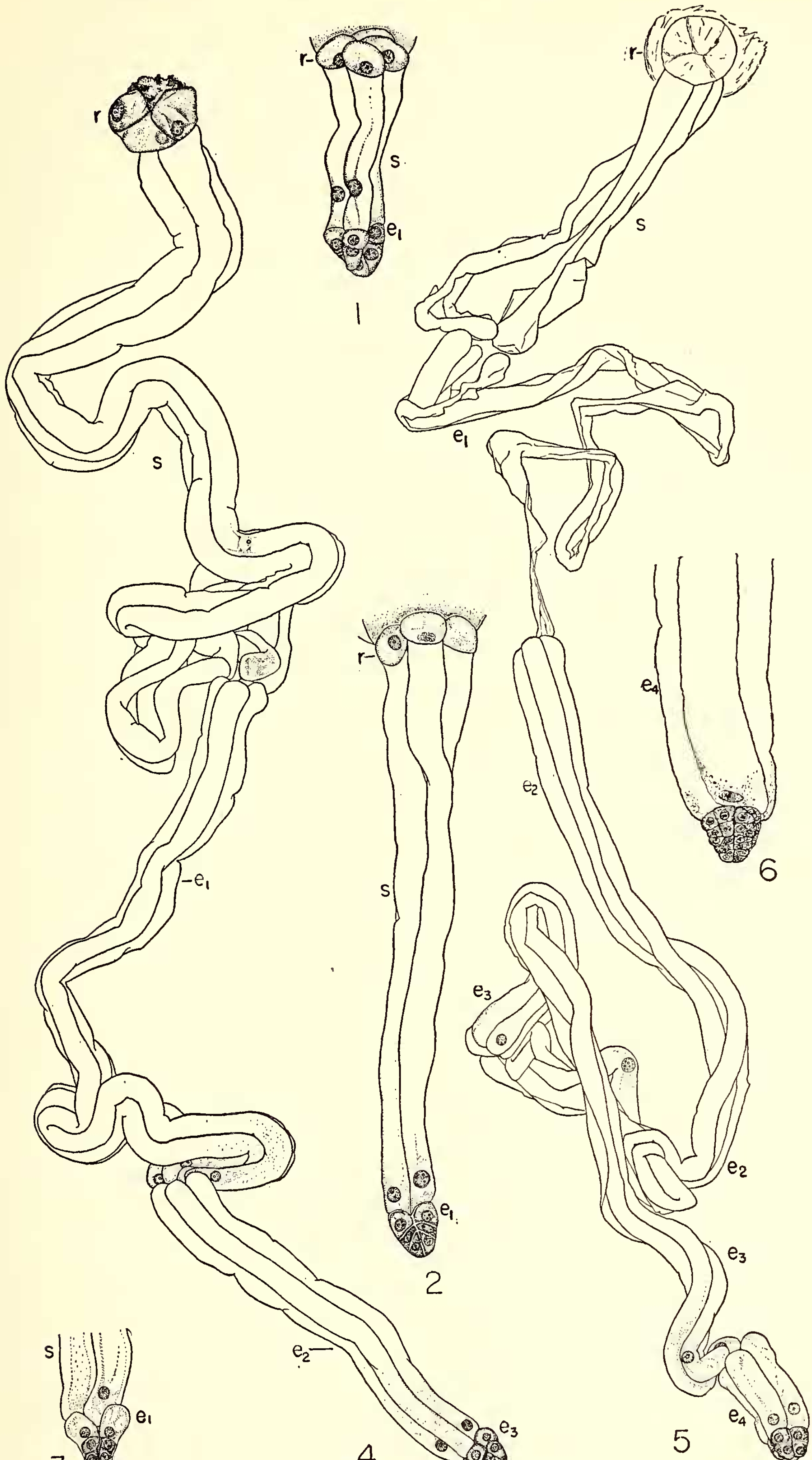

PLATE 15. SUCCESSIVE STAGES IN EMBRYOGENY OF PICEA SMITHIANA.

the four original embryonic cells as found in the lowest tier of the proembryo. In figure 3, the tier of cells next to the suspensor is beginning to elongate. This elongation is succeeded by that of the next tier, and by that of adjacent cells in tiers still to be added by the division of the terminal tier. Figure 4 shows a suspensor system in which the s, e_1 and e_2 tiers have elongated. For convenience the first tier is called the primary suspensor tier and the series of cells that elongate in successive 4-celled tiers may be designated as embryonal tubes e_1, e_2, e_3, and e_4. The e_4 is the last tier to be added to this regular succession before the terminal group of embryonic cells becomes more actively meristematic to increase greatly the number of cells in the entire embryo. The tiers that elongate subsequently to add the secondary suspensor come off in more irregular groups of cells. Figure 5 shows this stage in an embryo which has produced the e_4 sections of the suspensor. It is likely that the e_3 tier may at times be the last regular tier and in subsequent stages, the embryonal tubes lose their symmetry, become interlocked and more irregular in their elongation as they build up a massive secondary suspensor.

The writer can find no essential difference in appearance between the tiers s, e_1, e_2, e_3, etc., in *Picea*. There is probably no valid reason for making the distinctions implied by the names primary suspensor s and embryonal tubes of the successive orders e_1, e_2, etc., but this somewhat arbitrary designation is retained for the sake of uniformity and because it does seem to make a difference when compared with other genera of conifers. Figure 6 shows an embryo of more than 30 cells in which about 8 cells are adjacent to e_4 tier, which will elongate to form a much more massive addition to the suspensor.

The early suspensor system is, therefore, segmented and made up of four or five 4-celled and regularly tiered tubes. *Picea* has a rosette in which nuclei remain visible for some time, but the nuclei in the rosette disappear without undergoing division about the time the nuclei of the primary suspensor cells disintegrate. The successive suspensor cells collapse in the order of their age so that their remains and those of the rosette are sometimes difficult to find. The cells in the successive suspensor segments become about 20 to 30 times as long as their original diameter before the nuclei disappear and the suspensor elements collapse.

In *Picea,* simple polyembryony prevails. The fertilization of several eggs may give rise to several embryos which compete with each other, but the product of a single fertilized egg does not split up into several embryos, as in *Pinus, Cedrus* and *Tsuga.* The single embryo which survives usually has 8 to 10 cotyledons.

Picea Omorika (Pancic) Purkyne. The Siberian spruce was examined a number of years ago from trees planted at the Arnold Arboretum. The stages obtained were somewhat older than those described above, but the succession of tiered suspensor elements could also be observed in this species. Since the oldest elements had collapsed, it was not possible to be certain that e_4 was the last 4-celled tier that had elongated, though an e_3 tier could be recognized. In respect to the suspensor system and simple polyembryony, this Siberian spruce agrees with other spruces. The embryo as found in the mature seed of *Picea Omorika* had 5 to 6 cotyledons.

ABIES VENUSTA (Dougl.) K. Koch. The material for a study of the embryogeny of the Santa Lucia fir came from trees planted on the campus of Stanford University. The trees in the Santa Lucia mountains, their native region, were found to be too inaccessible and too far removed from a laboratory. Those on the grounds of Stanford University could be studied within an hour after collection. Their advantages were the early fertilization and the fact that the schedule of development fitted more conveniently into a research program which included other conifers.

The proembryo of no species of *Abies* has been adequately investigated. Miyake (6) observed only the free nuclear stages and Hutchinson (5) added a few stages in a study of *Abies balsama.* Hutchinson's account, especially his statement: "the proembryo of *Abies* ordinarily consists of eight cells only, in two tiers" is misleading and might leave one with the impression that *Abies* has a program in its proembryo which differs from other conifers. Actually it is very similar to *Pinus* (4) and *Picea* (7) in its early stages.

There is no doubt that the proembryo is the same as that described above for *Picea* except that the embryonic tier does not divide immediately. This tier divides only after considerable elongation of the suspensor tier.

The three tiers of cells—rosette, suspensor, and embryonic—are still present and undivided in *A. venusta* when the suspensor has elongated considerably. This is shown in figure 7 and in figure 8. Thus it is clear that there is really no serious hiatus in our knowledge concerning the proembryo of *Abies.* An opaque deposit has appeared over the rosette and all traces of the tier of relict nuclei have disappeared. In figure 9 each cell of the lowest tier of embryonic cells has divided so that this quartette of cells has now formed an octette arranged in two tiers. In figure 10 each of the original quartette of cells is represented by a progeny of four cells. The details of the embryo in figure 10 are shown better under higher magnification in two planes of focus in figures 10a and 10b.

The pointed tip of the embryo has a very thick hyaline wall which is also shown best in figures 10a and 10b. The pointed ends are very close together at first (figs. 7–9), become separated with the growth and lateral enlargement of the embryo, and remain as tiny projecting knobs that serve to identify the four derivative cell groups. These hyaline tips may be seen in successive stages shown by figures 11, 16, 17, and are obscurely recognizable in figure 18, in which the embryo has several hundred cells.

Sometimes the vertical rows do not contribute equally to the embryonic mass. Figures 12 and 13 show conditions in which inequalities have appeared in the development of the progenies of the 4 original embryonic cells. These do not show the small knobs that are seen in figure 17. In the latter, and in figure 18, the evidence is fairly conclusive that the cell progenies of the four original embryonic cells have contributed equally to the embryonic tissue.

Simple polyembryony prevails in *A. venusta* and is the normal condition in all firs that have thus far been examined. The product of each fertilized egg gives rise to a single embryo below the suspensor, and whether the four terminal embryonic cells contribute equally, as indicated in figures 17 and 18, or unequally, as indicated in figures 12 and 13, appears to make no distinguishable difference in later stages.

The suspensor *s*, composed of four cells, is remarkable in the amount of its elongation. Each cell elongates to about 60 times its original diameter before the terminal embryonic cells undergo their first division, and eventually becomes 150 to 200 times this length before the secondary suspensor begins to elongate, and before the primary suspensor cells collapse. Figure 16 has a suspensor in which each cell is fully 200 times as long as the width of these cells in figures 7 and 8. Figure 17 has suspensor cells that are not quite as long, but the ratio is fully 160 : 1. The latter has probably ceased elongation for the nuclei are no longer visible.

The secondary suspensor is formed by the elongation of cells (usually spoken of as embryonal tubes), from the distal end of the embryo, as shown in successive stages by figures 12, 13 (pl. 16) and 16–20 (pl. 17). As the enlarging embryo becomes wider, the number of elongating cells increases (figs. 13, 18), so that the secondary suspensor now becomes increasing'y more massive. The suspensor system may become greatly coiled and twisted. In dissections, some of these twists were removed, and those that are shown in the figures are not necessarily the ones that were most extreme before dissection. The newest additions of the secondary suspensor elongate most rapidly. These projecting newly-

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Explanation of the Figures. Plate 16.

Plate 16. Stages in Embryogeny of Abies venusta. Figs. 7–8, earliest stages dissected show 12 cells of 16-celled proembryo still undivided. Fig. 9, lowest (embryonic) tier of cells has divided. Fig. 10, later stage after four embryonic cells have each become 4-celled, with details of embryo shown in surface view in 10a and in lower focus in 10b (×147). Fig. 11, later stage with 8 cells in each of the four embryonic cells. Fig. 12, formation of secondary suspensor by elongation of cells from distal end of embryo. Fig. 13, later stage of same, showing rosette embryos derived from rosette cells, and well developed secondary suspensor with the suspensor cells now irregularly interlocked. Figs. 14–15, rosette embryos from embryo systems of stage similar to Figs. 12–13. All figures, except 10a and 10b, × 60. *r* = tier of rosette cells; *s* = primary suspensor; *e* = tier of embryonic cells.

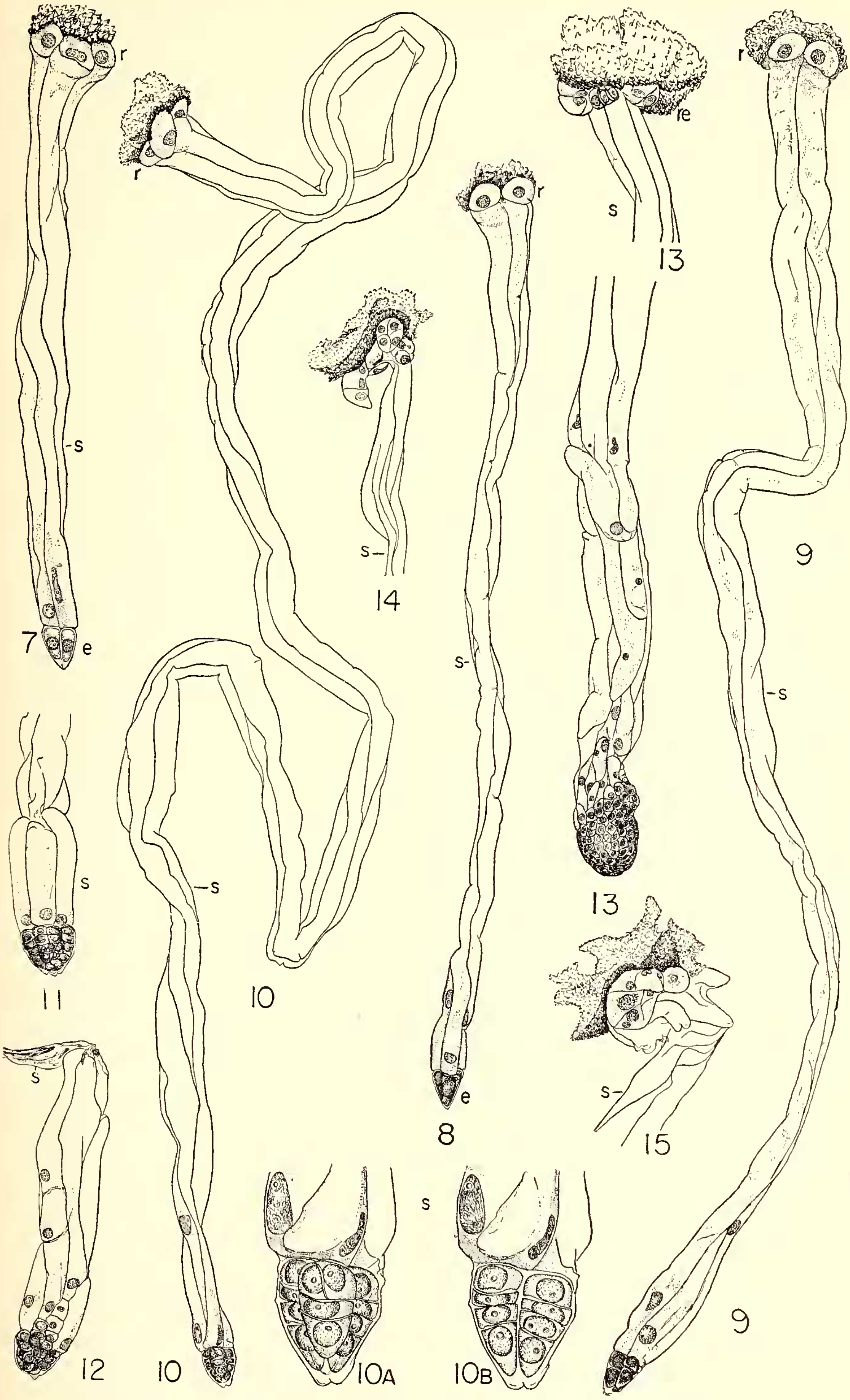

PLATE 16. STAGES IN EMBRYOGENY OF ABIES VENUSTA.

formed embryonal tubes usually ensnare any shorter embryos from another zygote that may be situated behind them and push the shorter rivals back toward the region of the archegonia.

Abies venusta usually forms rosette cells. A majority of these form rosette embryos, as may be seen in the upper part of figure 13 and in figures 14 and 15. These secondary embryos are formed only after considerable delay, during which the primary suspensor becomes fully elongated. In early stages shown by figures 7 to 10, and even in figure 16, the rosette cells usually remain undivided. In figure 17, each of the two rosette cells has divided once. Figures 14 and 15 were taken from embryos that had not only fully-elongated and collapsed primary suspensors, but the embryos had become massive and had developed secondary suspensors similar to those shown in figures 18 and 19. It was also observed that sometimes these rosette cells may fail to divide; the nuclei may disintegrate and the cells collapse without forming embryos.

The later stages of the successful embryos are shown in figures 19 and 20. *Abies venusta* agrees with other members of this family of conifers in that the primordium of the stem tip appears as a slight protuberance before the cotyledonary primordia are formed.

The embryo shown in figure 20 had 7 cotyledons surrounding and obscuring the primordium of the stem tip. The cotyledon number varied between 5 and 8. In a count of about 150 embryos, the mean number of cotyledons was 6.5.

The embryo of figure 20 has a conspicuous calyptroperiblem. This structure, which replaces the root cap in conifers, merges with the secondary suspensor and surrounds the plerome of the root tip. A ridge surrounding the embryo of figure 20 just below the middle marks the outer margin of the calyptroperiblem. In respect to this feature, the embryo of *A. venusta* resembles that of *Cedrus* (3) more than that of *Picea*.

ABIES PINSAPO Boiss. Embryological material of the Spanish fir was obtained from reproductive specimens found on the Mills Estate at Millbrae and on the Flood estate near Menlo Park, both in California. Only the early stages in the embryogeny were satisfactory. In stages later than those shown in the accompanying figures (21–24), the embryos were all aborted and the entire gametophytes shriveled up so that no viable seeds were matured. In the early stages there was a remarkable similarity with the embryogeny of *A. venusta*.

EXPLANATION OF THE FIGURES. PLATE 17.

PLATE 17. LATER STAGES IN EMBRYOGENY OF ABIES VENUSTA. Figs. 16–17, embryo systems showing extreme length of suspensor in a stage subsequent to Fig. 10, ×60. Fig. 18, embryo on well-developed secondary suspensor (stage following Figs. 11–13), ×60. Fig. 19, stage showing primordium of stem tip, ×20. Fig. 20, embryo after cotyledonary primordia have formed, ×20. *r* = rosette cells; *s* = primary suspensor; e_1 = beginning of secondary suspensor.

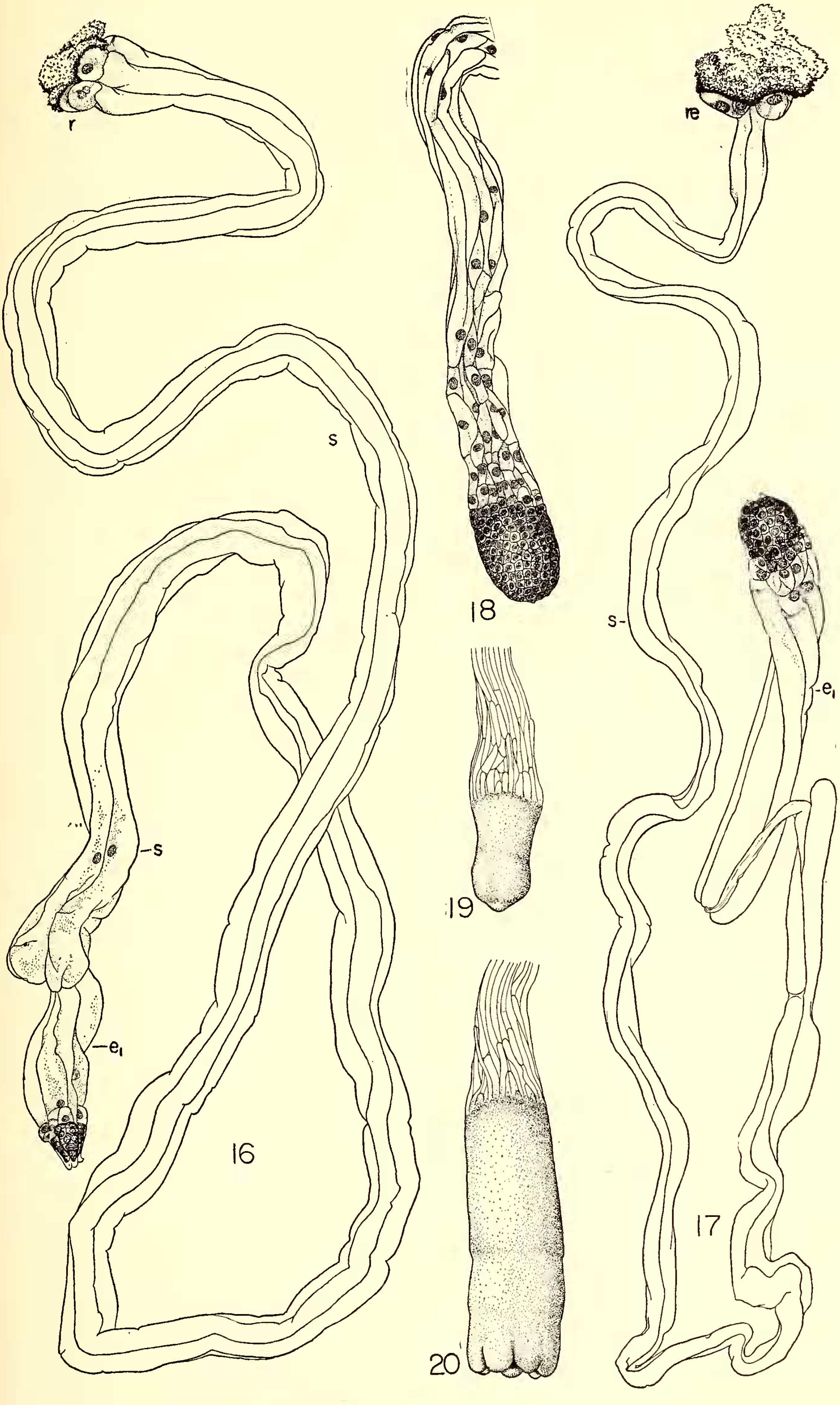

PLATE 17. LATER STAGES IN EMBRYOGENY OF ABIES VENUSTA.

Discussion

In the writer's earliest investigations on polyembryony (1), where the similarity in *Abies* and *Picea* with respect to simple polyembryony was stressed, the differences in the suspensor systems of these two genera was entirely overlooked. Unfortunately, these diagrams (copied by several authors) have been taken too literally. Much greater accuracy was achieved in a later publication (2), but in the second set of drawings, the rosette cells had usually collapsed in the embryos with long suspensors—those that were selected for the illustrations.

In re-examining these preparations, it is obvious that the single tier of primary suspensor cells of *Abies balsamea* collapses earlier and does not become as extreme in its elongation as the same structure in *A. venusta.* The difference between the spruce and fir is apparent in these old preparations and in the drawings made in 1930, which are fairly accurate, but the rosette cells and the older parts of the suspensor elements are so shriveled that they are very difficult to recognize.

Another feature which makes the rosettes and suspensor structures very difficult to study is not only the deposit shown above the rosette in nearly all of the figures, but a similar gummy or resinous deposit which surrounds the upper region of the suspensor system, omitted in all of the drawings.

There were several objectives that motivated this investigation. It was suspected that the embryogeny might offer features which would serve to separate *A. venusta* from the remaining firs and place it, either in one of the other genera or in a new genus. It was also desirable to discover the basis for a clearer definition of the differences between the genera *Picea* and *Abies,* both of which have simple polyembryony.

The taxonomic differences between *Abies venusta* and other firs are very marked. There are differences in the leaves, winter buds, bracts of the cone scale, and in the fact that the branches of this species are somewhat flexible and do not hold the cones erect as in other firs. The seed cones are inserted at nearly right angles on the twigs that bear them, are erect in early stages, but later their weight bends the slender twigs downward, so that many cones appear to be borne in horizontal positions, sometimes even drooping downward. The external differences may not be of great importance, but some of these characters such as the large peculiar winter buds and the very long, pointed leaves, so different from other firs, may have caused botanists to wonder whether this species may not represent something other than a true fir. In fact, several botanists, in discussing the unique external features in the Santa Lucia fir expressed the wish that the embryogeny be investigated in the hope that this might contribute new facts that might possibly prove to be decisive. *Abies Pinsapo* offered many external taxonomic features of contrast with *A. venusta.* How-

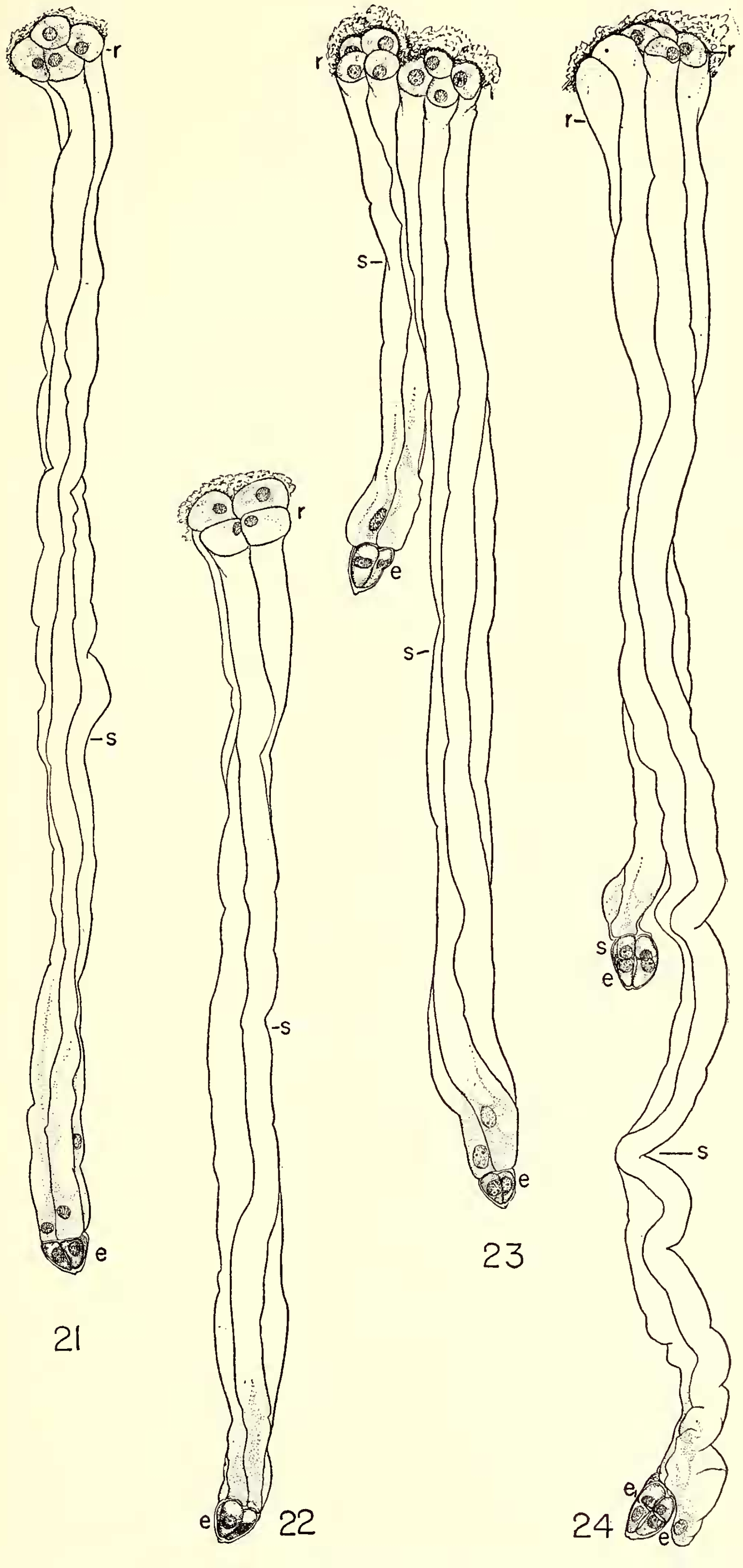

Plate 18. Successive Stages in Embryogeny of Abies Pinsapo. The paired embryo systems, Figs. 23–24, were dissected from the same ovules, × 60. *s* = primary suspensor; *e* = tier of embryonic cells.

ever, the embryogenies of these two species are in close agreement in the early stages that were observed.

Picea and *Abies* have a similar type of proembryo and both have simple polyembryony. Even though the proembryo of *Abies* has not been fully investigated, the three tiers of cells that remain are still undivided in the earliest stage shown in figure 7. The two genera differ, however, in the manner in which the suspensor system develops. In *Picea*, the primary suspensor tier (s) is followed by a succession of three or four similar additions, e_1, e_2, e_3, and e_4 before the secondary suspensor becomes irregular and may consist of many cells; in *Abies* a very long primary suspensor is formed which is directly succeeded by a more irregular many-celled secondary suspensor.

In *Picea*, rosette cells are formed but they usually abort and have not been observed to develop into embryos. In *Abies balsamea* and *A. Pinsapo*, these rosette cells usually fail to develop as in *Picea*, but in *A. venusta* a considerable number of instances were observed in which rosette cells developed into very small embryos.

Abies venusta agrees on the whole with the embryogeny of other species in this genus. It differs not only from *Picea* but also from *Pseudotsuga* (1, 2), in which no rosette cells are formed. *Abies venusta* differs from *Pinus*, *Cedrus* and *Tsuga*, all of which have cleavage polyembryony. There is no embryological feature in *A. venusta* which would tend to segregate it from the genus *Abies* save the rosette embryos which develop in a little more than half of the embryos. Other embryological characters that are distinctive in this species are quantitative in nature. The embryology of *A. venusta*, therefore, offers little in support of the idea that this species should belong to another genus. It definitely does not belong to any of the other genera now recognized and does not show embryological features which are sufficiently unique to suggest its segregation into a new genus.

Summary

1. The early embryogeny of *Picea Smithiana* is described with special reference to the development of the suspensor system.

2. The embryogeny of *Abies venusta* is described and compared with other firs, including *A. Pinsapo*, and with *Picea*.

3. Both genera begin their development with the same type of proembryo. In *Picea* the embryonic cells divide early and form three or four additional tiers of cells that elongate successively in adding relatively short tiers of elongated sections to the suspensor system. In *Abies* the embryonic cells do not divide until the suspensor tier has become well elongated. The suspensor cells elongate as a single tier, eventually becoming as long as the 3 to 4 tiers of additions in *Picea*, or even longer.

4. Rosette cells are formed in both genera. In *Picea* and some species of *Abies* the rosette cells do not divide to form em-

bryos; in *A. venusta* the rosette cells may form embryos after the suspensor tier has become fully elongated.

5. On the whole, the embryogeny of *Abies venusta* agrees closely with other firs. It does not belong in one of the other genera of Pinaceae and there seems to be little in the embryogeny which would suggest the segregation of *A. venusta* into a distinct genus.

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ANATOMY AND ECOLOGY OF AMMOPHILA ARENARIA LINK

Edith A. Purer

During a period of three years, studies were made upon sand dune plants along the Pacific coast from Oregon to Baja California. Environmental factors were measured and a study of the anatomy of twelve species was undertaken. In the subsequent publication (Purer, Studies of Certain Coastal Sand Dune Plants of Southern California, Ecol. Monog. 6: 1–88, 1936) the genus *Ammophila* was omitted since it was not abundant in the particular areas where the instrumental work was carried on.

Although it is not a native species, *Ammophila arenaria* Link has been successfully planted as a sand binder in a number of areas and is fairly well distributed along the Pacific coast. Specimens were examined at the herbaria in the following institutions, Stanford University (D); University of California (UC); and the University of Southern California (USC): Linnton, Oregon, September, 1927, *Thompson 3881* (D); Eureka Peninsula, Humboldt County, June, 1899, *Dudley* (D); Point Arena, Mendocino County, August, 1899, *Davy and Blasdale 6046* (UC); one-half mile south of Lake Merced, San Francisco County, May, 1901, *Dudley* (D); Jazos Creek, San Mateo County, March, 1922, *Bacigalupi* (D);